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SAMPLING POPULATIONS

OF WESTERN SPRUCE BUDWORM

AND

PREDICTING DEFOLIATION

ON DOUGLAS-FIR IN EASTERN
OREGON

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SUMMARY

Studies on sampling the western spruce budworm in eastern Oregon had the aim of developing a method for predicting defoliation of current growth of Douglas-fir, and applying this method in areawide surveys. Budworm density in nonfeeding stages was tested for predicting density of larvae in buds and this in turn, for predicting defoliation. As a shortcut approach, egg mass density was tested as an index to defoliation of current growth. The cost factor was given appropriate emphasis in selecting sampling units and in determining size and allocation of samples for efficient surveys.

Timing of sampling was compared with that for spruce budworm in eastern Canada. The sampling universe was a stratum of Douglas-fir dominants and codominants in second-growth stands in the Blue Mountains of Oregon. Mechanics of sampling involved collection of whole branches by climbers and collection of 15-inch twigs with a 35-foot pole-pruner. Distribution of sampling units on trees in the designated stratum was determined.

The egg stage and the larvae in buds appeared to be particularly suitable for sampling. A whole branch at midcrown was the sampling unit for the egg stage, and four 15-inch twigs from the lower half of the crown constituted the unit for larvae. Multistage analysis, involving variance and costs, was used to determine optimum size and allocation of samples at these life stages.

A cluster design appeared to be the best solution for sampling these stages. For low, medium, and high populations, number and size of clusters per stand and number of stands were determined for means with an acceptable sampling error of 20 percent, at $p = 0.05$, and also other precision and confidence levels. Sampling requirements were particularly great for low egg populations and medium populations of larvae in buds.

Prediction of current defoliation was shown possible. Tables were developed which use regression relationships to show egg mass density, corresponding density of larvae in buds, and expected degree of defoliation. Opportunities exist for expanding these tables; density of pupal cases and density of hibernating larvae showed promise in predicting density of larvae in buds. Techniques in estimating defoliation are believed to be a critical factor in setting up standards for prediction. We recommend fieldglass estimates, supplemented by periodic examination of foliage samples.

Keywords: Western spruce budworm, *Choristoneura occidentalis*, Douglas-fir, defoliation, sampling.

INTRODUCTION

Studies of the western spruce budworm, *Choristoneura occidentalis* Freeman, were initiated in 1950 to develop methods for sampling appropriate life stages and estimating visible damage. The primary objective was to develop efficient means of predicting budworm trends and damage. The study area was the Blue Mountains of eastern Oregon which contained epidemic infestations of the budworm from 1946 until 1959.

By 1950, studies on the eastern or spruce budworm, *C. fumiferana* (Clements) Freeman, were already underway in eastern North America. In New Brunswick, Canada, the Green River project had embarked on a long-term study of spruce budworm epidemiology in relation to forest management with preparation of life tables as the primary objective (Morris and Miller 1954). These studies were based on sampling absolute populations; population was expressed in terms of a basic unit, branch surface, and an absolute unit, the acre (Morris 1955). In New York and Maine, concurrent but separate studies were being conducted on factors related to budworm epidemiology. For the most part, these studies dealt with density of populations on a 15-inch twig basis (Dowden and Carolin 1950).

All sampling efforts have their limitations; and our approach was eventually a compromise dictated by a small work force, a number of susceptible host species, and biological characteristics of *C. occidentalis*. In Western United States, at least 14 species of conifers are acceptable hosts (Carolin and Honing 1972), and in the mixed types common to the Blue Mountains, four often occur in the same stand. The principal hosts--those on which greatest economic damage occurs--are Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) and grand fir (*Abies grandis* (Dougl.) Lindl.). On these hosts, in comparison with the eastern budworm on balsam fir, western budworm populations deposit larger egg masses, hibernate further inside the crown, and show greater diversity in age distribution of feeding larvae.

During the period 1950-54, most of our efforts were to determine proper timing of sampling according to biology of the insect and to assess our capabilities of obtaining and processing various sample units. At the same time, we attempted restricted population studies. From 1955 to 1959, special studies and routine sampling at semipermanent study plots were combined to assess sampling needs for predicting population changes on Douglas-fir. In presenting background and results of the various studies, we are following to some extent the format of Morris (1955), to which we refer the reader for concepts in sampling spruce budworm and other defoliator populations. Our presentation is concerned mostly with development of survey techniques; however, information on distribution of foliage and insects is particularly relevant to research studies.

CONSIDERATIONS IN SAMPLING

Timing of Sampling

Timing of sampling is aimed at separating an insect's life cycle into sampling periods so as to show effects of different mortality factors; it considers habits of the insect, life cycles of biotic control agents, and incidence of effects of physical environmental factors. Sampling at a time when population distribution is changing or when effects of specific control factors are incomplete should be avoided.

Sampling periods of possible use in sampling *C. fumiferana* were described by Morris (1955) as: eggs, second-instar larvae in hibernacula, second-instar larvae in mines, third-instar larvae in opening buds, fourth- to sixth-instar larvae, pupae, and moths. Two periods have been somewhat redescribed to fit *C. occidentalis*. A simplified term, "larvae in opening buds," is used to indicate a substantial spread in instar development in this period; and "fifth- to sixth-instar larvae," to designate the remaining period up to pupation. Eggs, and larvae in opening buds were periods of stability, as noted for *C. fumiferana* by Morris (1955).

Eggs

The period of egg deposition occurs in late July and early August and usually lasts about 3 weeks, with individual egg masses hatching in about 10 days. Use of hatched egg masses as a record of initial density and survival of the new brood is enhanced by persistence of the egg masses on the foliage. Initial evidence of this persistence was obtained in late spring of 1950 when an examination of either three or four midcrown branches from each of 11 codominant Douglas-fir trees yielded 1,146 egg masses, mostly well preserved. In subsequent studies, a method was developed for using old egg masses as an index to the previous year's egg population (Pacific Northwest Forest and Range Experiment Station 1958, p. 10-11; Buffam and Carolin 1966).

Timing of sampling was aimed at about 90-percent egg hatch. Because moth flight as well as other seasonal phenomena is extended by drastic local altitudinal and exposure differences, this timing insured that most eggs would have been deposited prior to sampling. We preferred to minimize an error from faulty timing, while accepting an increased risk of error in separating old and new masses.

Second-instar Larvae in Hibernacula

Larvae spend about 9 months hibernating among lichens and under bark scales on limbs and boles of the tree. Their tiny hibernacula are difficult to find; but after February 1, the larvae will issue readily to light in a warm room. Control personnel have collected and boxed limb and bole sections and have drawn larvae to the light to provide estimates of overwintered populations.

Sampling during a period from February 1 until late April is presumably satisfactory for estimating budworm populations surviving fall dispersal, predation, and unfavorable weather conditions. We found substantial predation on hibernating larvae to occur only in late summer and fall.

Second-instar Larvae in Mines

Second-instar larvae, after leaving hibernacula during May, usually mine into old needles and also staminate flowers if these are present. Residence in needle-mines may be as long as 10 to 14 days, and in staminate flowers up to 30 days.^{1/} Larvae in needle-mines molt into the third instar, and in staminate flowers, continue their development until the flowers dry out. Reliable estimates of budworm density during needle-mining are very difficult to obtain because of very gradual departure of larvae from hibernation; late-issuing larvae usually go directly to opening buds. In 1951, comparison of consecutive population estimates on foliage units showed up to twice as many larvae found in the buds as were previously found in mined needles. This finding discouraged any further sampling at this life stage.

Larvae in Opening Buds

After the larvae enter the buds, a period of about 10 days of relative stability occurs in which a single-host species can be sampled. Most buds have burst, but some buds remain swollen. At this time, larvae are mostly in third-, fourth-, and fifth-instars, with half or more in the fourth-instar. The diverse development apparently reflects the slow exodus of larvae from hibernation. Weather is normally cool during this period, particularly at night, so larvae develop slowly. Another factor in stability is low mortality during this period; 5 percent or less of the larvae are killed by parasites, predators, and other factors.

Differences in bud-bursting time between grand fir and Douglas-fir make it difficult to sample both tree species on the same date. A chart of bud development prepared during control studies in eastern Oregon in 1948^{2/} showed buds on grand fir to burst about 7 days earlier than those on Douglas-fir. Subsequent observations have indicated the difference often to be as great as 10 days.

Fifth- and Sixth-instar Larvae

This is a period of 20 to 30 days during which shoots complete their expansion. Biotic agents of control are particularly active, and careful timing is needed to obtain meaningful samples. Three hymenopterous parasites which

¹V. M. Carolin. Unpublished report. Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, New Haven, Conn., 1950.

²C. F. Speers. Annual progress report—calendar year 1948. Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, New Haven, Conn., p. 36, 1949.

have attacked second-instar larvae the previous fall issue from their budworm hosts and form cocoons during this period. At about the same time, other parasites commence to attack large larvae. Although a few minor parasite species attack and kill host larvae, four major species--all tachinids--have a delayed effect. Two of these, *Ceromasia auricaudata* Townsend and *Omotoma fumiferanae* (Tothill), issue as maggots several days after their hosts pupate; a third, "*Phorocera*" *incrassata* Smith, forms its puparium inside the host pupa; and a fourth, *Madremyia saundersii* Williston, issues from either larvae or pupae, but usually from the latter. *C. auricaudata* and *M. saundersii* are usually the most important species (Carolin and Coulter 1959). Therefore, to allow for full parasite attack, sampling should be timed with the early part of host pupation, and pupae are considered to be larvae, for purposes of estimating parasitism.

In 1950, the rate of change in parasitism in relation to percent of host pupation was assessed by daily collections of 1,600 to 3,700 budworm larvae and pupae. During an 8-day period in which host pupation increased from 9 to 55 percent, daily estimates of percent parasitism by individual species and all species combined, showed little change; they were uncorrelated with percent host pupation. Subsequently, timing has been aimed at 25- to 40-percent pupation as a compromise between allowing time for parasite attack and avoiding loss of *M. saundersii* maggots from larvae and fresh pupae.

Pupae

This is a short period of 14-16 days; hence, maximum time must be allowed for exposure of pupae to predators, particularly birds, and true pupal parasites. The latter, which both attack and emerge from pupae, include three ichneumonid and one sarcophagid parasite species. Tachinid parasites previously attacking larvae are not considered as pupal parasites.

To allow tachinid parasites to abandon pupae, we timed sampling for pupae with 60- to 90-percent emergence of the budworm, as indicated by abandoned pupal skins which remain fastened to silk webbing on the foliage. With this timing, pupae are seldom abandoned by true pupal parasites; but in rare cases where this occurs, the departed parasite can be identified by the type of emergence hole in the pupa.

Since true pupal parasitism in Oregon infestations is invariably low, sampling for pupae provides a good basis for estimating the size of moth populations before flight and mating.

Moths

Methods for measuring numbers of moths during the flight period had not been devised during these studies. The use of sex-attractant traps, now that a chemical attractant mutual to *C. fumiferana* and *C. occidentalis* has been identified and synthesized (Weatherston et al. 1971), offers promise in future sampling.

Sampling Universe

The concept of a limited universe as defined by Morris (1955) was used in these studies. A small area or stand of about 20 acres characterized by homogeneous conditions was recognized as the universe. Also, since our objective was not to measure absolute populations, one stratum was designated for sampling.

The designated stratum was Douglas-fir dominants and codominants, 50 to 85 feet tall, a common height class in second-growth stands of eastern Oregon. Douglas-fir was selected in preference to grand fir because of: (1) its greater abundance on a variety of exposures and sites, (2) its higher economic value, and (3) its apparent ability to withstand budworm feeding over a period of years without severe reduction in the number of growing tips. Major substrata from which representative samples might be drawn were thirds of the tree crown.

The sampling problem relating to grand fir was gradually explored by paired tree comparisons between Douglas-fir and grand fir as opportunity presented itself. These results will be reported in another paper.

Mechanics of Sampling and Sampling Units

Various units were tested for sampling specific life stages, and the mechanics of obtaining these units varied. Only the methods used for hibernating larvae differed appreciably from those already devised for the eastern budworm.

Bark samples for second-instar larvae in hibernacula were obtained from both limbs and boles of trees. Limbs were cut from either felled or standing trees and sawed into sections 14 or 15 inches long. Bark of boles was obtained by either felling trees and sawing 14-inch bole sections or cutting bark strips from standing trees by means of a large curved knife. Limb and bole samples were placed in cardboard boxes equipped with shell vials which were provided with continuous light. Larvae entering the vials were removed and counted twice daily.

Foliage samples, including the 15-inch twig, whole branch, and foliated branch area, were tested for other life stages. The 15-inch twig was defined as any twig 15 inches long, or the apical 15 inches of a longer twig, and bearing at least one living bud. Measurement was from the base of the terminal bud or expanding shoot. Branches on trees in the designated stratum were generally large; average foliated length of midcrown branches was about 6-1/2 feet. Terminal sections of branches, such as the 24-inch branch (McKnight 1968), were not tested in these studies nor was the branch tip method described by Wilson (1959).

A six-section aluminum pole-pruner with a total length of 35 feet was used to remove 15-inch twigs from sample trees. Maximum height of sampling was about 40 feet if the pruner were held at chest height. Different

numbers of sections were used so as to sample different parts of the crown at random. The pole-pruner was equipped with an iron-rimmed basket, 18 inches in diameter and having a muslin net. The twigs clipped into the basket were normally examined on a mat in the field as sampling proceeded. Usually, two men operated pole-pruners and two men examined twigs for budworm. A modified tree pruner which both cuts and holds twig samples has been described by Stein (1969).

When we climbed trees to remove whole branches, a 24-foot aluminum ladder was used to gain access to the lower branches of trees; branches were removed with hand snips or pruning saw. When larval populations were sampled, branches were carefully passed down the tree from one climber to another. When egg populations were sampled, branches were thrown clear of the crown so as to land on a large canvas mat. Branches taken to sample feeding larvae were examined on a cloth in the field. Branches taken to sample egg populations were carried intact back to the field laboratory. Each branch was then cut into two piles consisting of 15-inch twigs and remaining foliage. A crew of four women clipped the foliage in each pile into smaller pieces and removed all insect material. The crew chief separated egg masses from extraneous material and placed both types of material in individual triple 0 gelatin capsules. He listed insect material by branch on a standard form and periodically checked foliage as it was discarded. Later, a scientist examined the material and separated egg masses into "new" and "old" categories.

Foliated branch area was determined by the method of Morris (1955), with a modification to accommodate the irregular shape of Douglas-fir branches. The modified method involved measuring foliated length and greatest foliated width (rather than width at midlength) and calculating the area as two triangles. Numbers of budworm were expressed on the basis of 1,000 square inches. If a branch forked, foliated area of each of the forks was calculated separately and the sum of these areas determined.

Vertical Distribution of Foliage Sampling Units

Numbers of foliage sampling units were estimated on codominant Douglas-fir trees to determine their proportion among crown thirds, variation in total number between trees, and any biological significance associated with these units.

Methods

Units studied were the whole branch, foliated branch area, and the 15-inch twig. Fifteen trees ranging from 53 to 78 feet in height were felled, then their crown lengths were measured and divided into thirds. Number of living branches was counted, and each was designated as primary or internodal. Starting at the base of the crown, we removed every fourth branch, measured its foliated branch area, and counted the number of 15-inch twigs. Total foliated area and total number of 15-inch twigs in each crown third were obtained by applying the ratio of total number of branches counted to number of branches sampled.

We hypothesized that the distribution of foliage (foliated branch area and number of 15-inch twigs) would approximate that for the surface of a right circular cone. If so, foliage would be distributed in the following proportion: upper third, 1; middle third, 3; and lower third, 5. An alternative was to determine foliage distribution by regression analysis. However, conformity to the distribution for a right circular cone would have an important implication: If numbers of budworm were approximately the same in each crown third, average numbers per unit of foliated branch area would always be found within the middle third. Thus, sampling for purposes of trend would be greatly simplified. Also, a known distribution of foliage between crown thirds would provide a means for weighting estimates of defoliation by crown third to obtain a single defoliation estimate for the tree.

Results

Number of primary branches did not differ significantly among crown thirds, but number of internodal branches was much higher in the upper third, approximating the number of primary branches, than in the other crown thirds (table 1). Thus, if whole branches are used to sample the various crown thirds, particular care must be taken in the upper third to avoid a biased selection.

Table 1.--*Vertical distribution of branches on Douglas-fir trees near Baker, Oregon, 1955*

Tree number	Number of primary branches by crown third				Number of internodal branches by crown third			
	Lower	Middle	Upper	Total	Lower	Middle	Upper	Total
1	39	40	54	133	0	0	28	28
2	51	48	58	157	0	8	24	32
3	36	42	67	145	0	0	12	12
4	36	38	40	114	0	4	40	44
5	47	52	44	143	0	16	84	100
6	45	47	52	144	0	8	36	44
7	40	40	54	134	4	8	24	36
8	52	47	44	143	0	0	52	52
9	55	50	32	137	8	16	44	68
10	49	47	53	149	0	16	48	64
11	34	39	50	123	0	4	40	44
12	58	61	60	179	4	16	52	72
13	35	27	48	110	12	12	24	48
14	50	39	38	127	4	12	72	88
15	40	42	46	128	0	12	40	52
Total	667	659	740	2,066	32	132	620	784
Average	44.5	43.9	49.3	137.7	2.13	8.80	41.33	52.26

After exclusion of one open-growing tree, which had an abnormally large amount of foliage for its d.b.h. (diameter at breast height), foliated branch area was distributed among the crown thirds as hypothesized, with no significant difference between the observed and expected ratios (table 2). There was a highly significant ($p < 0.01$) linear correlation between foliated branch area and d.b.h. ($r = 0.88$).

On the other hand, observed proportions of 15-inch twigs differed significantly ($p < 0.05$) from expected values. With the single open-growing tree excluded, the only significant difference was between the middle and lower thirds. There were fewer 15-inch twigs in the lower third than expected, apparently reflecting loss of bud production and gradual dying of lowermost twigs. Total number of twigs on d.b.h. gave a linear correlation ($r = 0.89$).

These findings provide initial guidelines for representative sampling of codominant Douglas-fir in subsequent research efforts and a means for weighting defoliation estimates obtained by crown third, for use in biological evaluations.

OPTIMUM SAMPLE SIZE AND ALLOCATION AT TWO SAMPLING PERIODS USEFUL FOR PREDICTION

Two periods--eggs, and larvae in opening buds--offer particular promise in developing a method for predicting damage over large areas, in order to anticipate control needs. One approach is to develop techniques to predict larval counts from egg counts and then to predict foliage damage from larval counts. Two other periods--second-instar larvae in hibernacula and pupae--also offer some promise as a basis for predicting numbers of larvae in buds, but sampling techniques need further development. The basis for prediction is empirical and will be discussed later.

The first need was to develop methods for obtaining sample estimates of budworm density with an acceptable precision and at an acceptable cost. Sampling data were collected at plots separated by 35 miles or more, each plot in a relatively homogeneous stand condition extending between 15 and 25 acres. Each plot consisted of five subplots located five chains apart on a compass line. At each subplot, two trees were marked for egg sampling and five for larval sampling. Three or four plots were sampled each year; plots affected by spraying were replaced.

Egg Populations

Objectives were to determine: (1) whether significant differences in number of egg masses occurred between crown thirds, (2) relative efficiency of estimates for different sample units from different crown thirds, and (3) optimum size and allocation of samples for the middle crown third. Middle crown third was specified in objective 3 so essentially the same part of the crown would be sampled at successive life stages.

Table 2.--Vertical distribution of foliage units on Douglas-fir trees near Baker, Oregon, 1955

Tree number	D.b.h.	Height	Foliated branch area by crown third				15-inch twigs by crown third			
			Lower	Middle	Upper	Total	Lower	Middle	Upper	Total
			-----Thousand square inches-----				-----Number-----			
Inches			Feet							
1	12.7	63.0	66.4	56.5	14.3	137.2	400	352	72	824
2	16.4	78.0	105.0	72.2	22.4	199.6	676	596	172	1,444
3	12.7	76.5	62.1	54.2	15.8	132.1	400	324	84	808
4	13.0	66.5	70.0	48.4	19.7	138.1	296	396	140	832
5	14.5	73.4	79.7	48.4	14.5	142.6	552	356	80	988
6	12.8	54.2	77.2	66.1	20.5	163.8	556	468	124	1,148
7	11.6	63.6	43.7	35.6	12.5	91.8	268	280	92	640
8	13.2	69.2	87.3	53.8	11.3	152.4	412	368	84	864
9	11.7	56.2	112.2	91.8	22.4	226.4	680	688	120	1,488
10	11.6	67.8	44.2	27.8	10.3	82.3	316	224	80	620
11	12.7	62.0	65.0	42.5	11.0	118.5	416	248	68	732
12	10.7	68.3	36.9	23.8	9.1	69.8	356	220	68	644
13	9.9	53.3	56.4	27.9	7.7	92.0	328	140	48	516
14	8.8	53.0	41.2	22.6	10.8	74.6	304	140	80	524
15	9.8	55.1	47.1	31.4	14.0	92.5	284	204	76	564
Averages			63.0	43.7	13.8	120.5	397	308	91	796

1/ This tree was rejected as abnormal--for its d.b.h., both foliated area and number of 15-inch twigs are unusually large so are not included in averages.

Methods

Egg populations were expressed as number of egg masses; sample units tested for the different crown thirds were the whole branch and foliated branch area. In objectives 1 and 2, data were from two low population plots in 1955, one plot with a rapidly increasing population sampled from 1955 through 1957, and one high population plot in 1957. Two trees per subplot were sampled at the plot studied for 3 years and one tree per subplot at all other plots. One branch was removed and examined per crown third.

Differences in egg mass populations by crown thirds were tested by analysis of variance. At the 3-year plot, some sample trees were replaced during the 3-year period. Therefore, analysis was based on five trees used both in 1955 and 1956, and five trees used both in 1956 and 1957. In addition, all 10 trees used in 1956 were tested. At the other plots, analysis was for a single year.

Relative efficiency of sampling different crown thirds for egg masses was analyzed by the following criteria: (1) coefficient of variation of the mean among subplots and (2) costs of obtaining and examining samples.

Optimum size and allocation of samples for the middle crown third were determined from variation within and among subplots and from relative costs attributable to plots (stage 1), subplots (stage 2), and trees (stage 3), using a multistage computer program.^{3/} Optimization was initially based on a sampling error of 20 percent at $p = 0.05$. Other options used for comparison were: $SE = 0.10$ at $p = 0.05$ and $p = 0.10$; $SE = 0.20$ at $p = 0.10$; and $SE = 0.40$ at $p = 0.05$ and $p = 0.10$. Data consisted of 12 records from five plots during 1955-57. At each subplot, we sampled two trees by removing one midcrown branch from each tree and examining it for new egg masses. Plot-years having similar means were pooled so as to recognize three population strata--low, medium, and high. Following this analysis, allocation of resources for low populations was retested, using Region 6 survey data for plots with five trees--six plots in Douglas-fir stands and five in grand fir stands. Because two longitudinal half-branches per tree were taken at survey plots, the sample branch was selected by flipping a coin. The number of egg masses on the selected branch was then doubled.

Stratification of egg populations for multistage analysis was based on the following parameters, in terms of number of egg masses per one midcrown branch sample per Douglas-fir tree.

<u>Stratum</u>	<u>Mean</u>	<u>Range</u>
Low (four plots)	4.45	3.1-5.7
Medium (five plots)	9.24	7.1-12.1
High (three plots)	25.57	19.5-33.9

³ John W. Hazard and Larry E. Stewart. Choosing sample sizes in multistage sampling. (In preparation for publication, Pacific Northwest Forest and Range Experiment Station.)

The low population stratum for survey plots was essentially the same as for the research plots, using plot averages based on the two midcrown branch samples as a guide. However, the exercise in randomization caused the upper range on grand fir to infringe on the lower range of the medium category. Parameters in terms of egg masses per single midcrown branch for this stratum at the survey plots were:

<u>Species</u>	<u>Mean</u>	<u>Range</u>
Douglas-fir (six plots)	3.93	2.4-6.4
Grand fir (five plots)	4.92	3.2-7.2

Results

Differences among crown thirds.--When the whole branch was used, egg mass numbers were generally lowest in the upper crown third (table 3). However, significant differences were found only at the 3-year plot. In 1956, number of egg masses in the upper third was significantly lower than those in the middle third; in the 1956-57 comparison, numbers of egg masses in both the upper and lower thirds were significantly lower than those in the middle third.

When foliated branch area was used, density of egg masses was generally lowest in the lower crown third and highest in the upper crown third (table 3). A significant difference was found only in the 1956-57 comparison for the 3-year plot; egg mass density was significantly higher in the upper crown third than in the lower crown third.

These findings are not entirely conclusive. However, the middle third appears likely to provide average estimates for the tree, in terms of either number of egg masses or egg mass density.

Efficiency of different crown thirds.--The analysis of relative efficiency of different crown thirds for sampling showed variation of estimates to be a minor factor and costs a major factor. Coefficients of variation (*CV*) among subplots, for either the whole branch or foliated branch area, were not consistently lower or higher for any particular crown third. A slight advantage was gained from expressing egg masses on a branch area basis; for all plots, average *CV* for the whole branch was 0.631 and for foliated area, 0.623. At the 3-year plot, where two trees were sampled per subplot, *CV*'s were lower than at the other plots, 0.579 and 0.568, respectively.

Costs of collecting and examining branches were lowest for those from the upper third, with progressively increasing costs for middle and lower thirds. Differential costs of collecting branches, based on one man climbing

Table 3.--Vertical distribution of egg masses on sample branches from Douglas-fir in eastern Oregon

Locality	Years	Number of trees	Average population by crown position			Significant difference in population ^{1/}		
			Low (L)	Middle (M)	Upper (U)	Year	Crown position	Nature of difference
NUMBER OF EGG MASSES PER BRANCH								
Baker	1955-56	5	4.8	3.7	1.5	S ^{2/}	NS ^{3/}	1956>1955
	1956	4/ 10	6.2	8.5	3.2	--	S	M>U
	1956-57	5	4.3	10.6	5.8	NS	S	M>L, M>U
Chesninnus	1955	5	9.4	6.4	4.0	--	NS	--
Joseph	1955	5	6.0	7.4	3.4	--	NS	--
Dixie	1957	5	28.0	25.2	14.0	--	NS	--
NUMBER OF EGG MASSES PER 1,000 SQUARE INCHES OF FOLIAGE								
Baker	1955-56	5	3.2	3.1	3.5	NS	NS	--
	1956	10	3.4	5.7	7.2	NS	NS	--
	1956-57	5	2.6	5.8	10.4	NS	S	U>L
Chesninnus	1955	5	6.9	8.2	9.6	--	NS	--
Joseph	1955	5	3.9	7.8	4.5	--	NS	--
Dixie	1957	5	9.1	11.2	12.1	--	NS	--

^{1/} p = 0.05.

^{2/} S = significant.

^{3/} NS = nonsignificant.

^{4/} A separate analysis was made for 1956 because of significant increase in average number of egg masses.

and one man on the ground, were minor; differential costs of examination were major. A simplified breakdown of costs per branch in man-hours is as follows:

	<u>Upper third</u>	<u>Middle third</u>	<u>Lower third</u>
Collecting	0.8	0.6	0.4
Examining	<u>2.3</u>	<u>4.5</u>	<u>6.7</u>
Total	3.1	5.1	7.1

Samples from the upper crown third provide the most efficient basis for measuring levels of egg populations, if qualified climbers are available. However, if the objective of sampling requires estimates which are representative for the tree, one would sample the appropriate crown third and accept the higher costs involved. Development of a method of subsampling the branch would reduce costs and probably result in different relative efficiencies among crown thirds.

Optimum sample size.--Multistage analysis of sample counts and cost data for the middle third showed optimum sample size and allocation to differ between high or medium populations and low populations (table 4). In high and medium populations, variation among third-stage units (trees) is sufficiently small that only one tree should be sampled per subplot; it is efficient to take several subplots within a stand and sample several stands. For these higher population levels, six to eight plots, each consisting of eight or nine scattered trees, are near optimum at $SE = 0.20$ and $p = 0.05$ for sampling a specified area. In low populations, variation among third-stage units is very large relative to the other stages, and three-stage sampling is not an acceptable alternative. Instead, a 10-tree subplot should be the smallest unit of sampling. A two-stage analysis of egg-mass data from five-tree survey plots confirmed this conclusion. Ten-tree or larger clusters should be the secondary units to be selected from the number of possible such units per stand, and "n" stands should be selected as the sample of primary units. In eastern Oregon, groups of Douglas-fir in the specified stratum and accessible for sampling rarely exceed 10-12 trees.

Estimated number of plots for different levels of precision and confidence, excluding low populations, are summarized below.

<u>SE</u>	<u>p</u>	<u>Medium</u>	<u>High</u>
(Number of plots)			
0.10	0.05	24.3	30.4
.10	.10	17.1	21.4
.20	.05	6.1	7.6
.20	.10	4.3	5.4
.40	.05	1.5	1.9
.40	.10	1.1	1.3

Table 4.--*Optimum multistage sample sizes for sampling egg populations in eastern Oregon*^{1/}

Stage	Total number	Sample size	Sampling fraction	Sample variance	Estimated population variance	Cost limit (man-hours)	Estimated optimum sample size ^{2/}
HIGH POPULATIONS							
1 (plots)	9999 ^{3/}	3	--	55.69	29.73	16.0	7.6
2 (subplots)	75	5	0.0667	134.96	77.13	.4	9.0
3 (trees)	10	2	.2000	144.57	144.57	5.7	1.0
Estimate of mean 25.57				Specified variance of mean 6.81			
MEDIUM POPULATIONS							
1 (plots)	9999 ^{3/}	5	--	5.05	2.17	16.0	6.1
2 (subplots)	75	5	.0667	14.77	5.43	.4	8.0
3 (trees)	10	2	.2000	23.36	23.36	5.7	1.0
Estimate of mean 9.24				Specified variance of mean 0.89			
LOW POPULATIONS							
1 (plots)	9999 ^{3/}	4	--	1.67	0.82	16.0	1.0
2 (subplots)	75	5	.0667	4.09	-2.05	.4	2.0
3 (trees)	10	2	.2000	15.35	15.35	5.7	10.0
Estimate of mean 4.45				Specified variance of mean 0.21			

^{1/} Precision, 20 percent of mean at $p = 0.05$.

^{2/} Cost is minimized subject to specified variance of the estimated mean.

^{3/} Code for infinite number.

Various options on sample size may be selected within budgetary constraints; selection may be influenced by number and size of infested areas. Further, a higher priority for sampling is likely to be assigned to medium and high populations, as compared with low populations. If a single large area were to be sampled, one might prescribe $SE = 0.10$ and $p = 0.10$; sampling requirements would be about 17 clusters for medium populations and 21 for high populations. If several areas were to be sampled, one might prescribe $SE = 0.20$ and $p = 0.10$; sampling requirements per area would be four to five clusters for medium populations and five to six for high populations. Budgetary restraints would normally encourage sample sizes based on $SE = 0.20$ and either $p = 0.05$ or 0.10 , rather than higher precision. Higher sampling errors would be tolerable for low populations. Since multistage sampling appears to be an unacceptable alternative for low populations, we recommend only that 10-tree or larger clusters be sampled in several stands or locations. Variation among these clusters will determine the exact number of stands.

Larvae in Opening Buds

Objectives were to determine: (1) significant differences in numbers of larvae between crown thirds, (2) relative efficiency of 15-inch twigs and whole branches in sampling the same part of the crown, and (3) optimum size and allocation of samples according to trees, subplots, and plots. A major concern was the development of an efficient sampling method based on removal of 15-inch twigs with a pole-pruner, rather than on whole branches obtained by tree climbers.

Methods

Differences in numbers of larvae by crown third were tested in 1955, using six randomly selected trees at a plot having low populations. Three whole branches were removed from each crown third, and individual and total branch areas were determined for each third. All damaged buds were opened and total number of spruce budworm larvae recorded for each group of three branches. Covariance analysis of number of larvae and foliated branch areas was used to test for differences between crown thirds, after adjusting numbers of larvae to a common foliated area (average for all trees). The same analysis was run without adjustment of counts to a foliated area basis.

Relative efficiency of sampling by (1) 15-inch twigs removed by use of a pole-pruner and (2) branches obtained by climbers was determined in 1956 at a low-population plot, and in 1957 at a high-population plot. Sampling units were a four-twigg lot, taken from each of 25 trees, five per subplot, and either two branches (low-population plot) or one branch (high-population plot) from each of 10 trees, two per subplot. Sample twigs were cut at random from the lower half of the crown. Branch samples from the low-population plot were taken from the middle of the lower half of the crown and from the high-population plot at mid-crown. Larvae were counted on these samples, and number of man-hours required for sampling was recorded. Relative efficiency was based on a comparison of coefficients of variation and number of trees that could be sampled in a unit period of time.

Optimum allocation of sampling effort was determined for the method based on 15-inch twigs. Analysis was based on variance in numbers of larvae on four-twig lots at standard plots, and relative costs attributable to plots, subplots, and trees. Different population strata were recognized and separate analyses were made for each stratum. The multistage computer program by Hazard and Stewart (see footnote 3) was used again to determine the most efficient allocation of plots (stage 1), subplots (stage 2), and trees (stage 3).

A total of 18 plot records was examined in order to pool data into logical strata. Initial stratification was based on the plot mean (average number of larvae per four twigs). A second step was to reconcile the stratum for each plot with the stratum recognized during egg sampling the previous fall. Final recognition of the different strata was based on the following parameters, in terms of larvae per four 15-inch twigs:

<u>Stratum</u>	<u>Mean</u>	<u>Range</u>
Low (seven plots)	8.17	5.64-9.84
Medium (five plots)	13.27	11.24-17.40
High (four plots)	35.38	23.88-43.20
Very high (two plots)	77.72	68.00-87.44

Analyses were necessarily restricted to three strata. An additional run was made, combining high and very high populations.

Results

Differences among crown thirds.--Numbers of larvae did not differ significantly among crown thirds, either on a foliated branch area basis or on branches unadjusted for area. Before adjustment for branch size, mean numbers of larvae were highest in the lower third and lowest in the upper third. After adjustment, the order was reversed (table 5). Thus, vertical distribution of larvae in the buds is apparently no different from that of egg masses; for both life stages, the population is generally distributed over the whole crown. As a result, consecutive sampling for the two life stages appeared feasible to test predictive value of egg mass sampling.

Relative efficiency of two sampling methods.--Sampling larvae in the buds by the 15-inch twig method was more efficient than sampling by whole branches. Means of four-twig lots had higher coefficients of variation than did whole-branch means, but the cost of twig-sampling was much lower than that of branch-sampling. At the low-population plot, average costs per tree of obtaining and examining samples were 1.2 man-hours for 15-inch twigs, and 3.6 for whole branches (two branches per tree). At the high-population plot, the costs were 1.5 man-hours for 15-inch twigs and 5.7 for whole branches (one branch per tree). Examination of whole branches is costly, and costs increase rapidly with density of larvae.

Table 5.--Vertical distribution of larvae in buds on sample branches
from Douglas-fir trees near Baker, Oregon, 1955

Tree number	D.b.h.	Lower third of crown		Middle third of crown		Upper third of crown	
		Foliage area	Larvae	Foliage area	Larvae	Foliage area	Larvae
	Inches	Thousand sq. in.	Number	Thousand sq. in.	Number	Thousand sq. in.	Number
1	11.6	4.709	57	3.468	30	1.023	38
2	12.5	8.758	93	5.427	76	1.066	35
3	10.8	3.493	6	1.620	15	.597	2
4	10.1	5.721	32	3.810	37	1.227	25
5	8.9	3.495	45	2.282	36	.919	42
6	9.7	6.357	8	1.882	12	1.929	31
Total		32.533	241	18.489	206	6.761	173
Column means			40.17		34.33		28.83
Adjusted means ^{1/}			23.43		35.31		44.60

^{1/} Adjusted to average foliated branch area for all tree samples.

Analysis involved computation of relative sampling errors, using $SE\% = \sqrt{\frac{CV^2}{n}}$, with n the number of trees that can be sampled in a unit period of time. When 50 man-hours is used as the unit period, the following tabulation illustrates the difference in efficiency of the two methods:

Sampling unit	Low-population plot			High-population plot		
	CV	n	SE%	CV	n	SE%
Twig lots	0.57	42	9	0.51	34	9
Branches	.42	14	11	.47	9	16

Optimum sample size.--Optimum allocation of effort for sampling larvae in buds was found to vary with population strata, a situation also found in egg-mass sampling. In terms of range of sample estimates, the low-population stratum was narrow, but medium- and high-population strata were broad. A very high level was recognizable at one plot for 2 years. However, in first-year sampling, no more than two strata of higher populations, medium and high, can reasonably be considered.

To sample low populations, several clusters of 10 trees or more, as prescribed in egg-mass sampling--but in a very few stands--appear to be the most efficient approach. For $SE = 0.20$ at $p = 0.05$, the multistage analysis indicates six 10-tree subplots at each of two plot locations to be the approximate sampling solution (table 6). Broadening the concept of a plot location would permit random selection of 10-tree clusters from various nearby stands and would improve the general approach to sampling.

In the case of medium populations, components of variation of the second and third stage are too large to define satisfactorily a multistage problem, possibly because this stratum is in fact a mixture of low and high populations. The solution from analysis indicates that a very large number (i. e., 75) of clusters of 10 trees or more should be allocated to a single stand (table 6). This is unrealistic because homogeneous stand conditions are usually restricted to 15-20 acres. The best approach is probably to sample fixed clusters of trees scattered over a designated area, without reference to particular stands.

To sample high populations as defined, two clusters or subplots each consisting of 10 or more trees should be sampled at each of a few plots (table 6). For $SE = 0.20$ at $p = 0.05$, five plots are adequate. Pooling two plot records for very high populations with the four records for high populations resulted in substantially increased sampling requirements. For the same precision, about 19 plots should be sampled, using one cluster of 10 or more trees in each plot. Good criteria for preliminary recognition of very high populations would help avoid the combination of these two strata and thus conserve sampling resources.

Other levels of precision and confidence provide some options on sampling low and high populations, but not medium populations because these were characterized by very high variation. With low populations, accepting $SE = 0.20$ at $p = 0.10$ or $SE = 0.40$ at $p = 0.05$ leads to rejection of the multistage solution. The result, however, provides an option to utilize cluster sampling in several stands at each of very few locations as in egg-sampling. A higher precision ($SE = 0.10$ at $p = 0.05$) prescribes five plots with six 10-tree clusters per plot. With high populations, increased precision leads to a substantial increase in numbers of plots consisting of two 10-tree clusters per plot, with 18 at $SE = 0.10$ and $p = 0.05$, and 13 at $SE = 0.10$ and $p = 0.10$. Budgetary restraints would normally discourage this intensity of sampling, particularly if two or more areas were to be sampled, because of greatly increased costs associated with examining twigs when larval populations are high.

Estimated numbers of plots for different levels of precision and confidence, excluding medium populations, are summarized below.

<u>SE</u>	<u>p</u>	<u>Low</u>	<u>High</u>
0.10	0.05	4.9	18.1
.10	.10	3.4	12.8
.20	.05	1.2	4.5
.20	.10	1.0	3.2
.40	.05	1.0	1.1
.40	.10	1.0	1.0

Table 6.--Optimum multistage sample sizes for sampling populations of larvae in buds in eastern Oregon^{1/}

Stage	Total number	Sample size	Sampling fraction	Sample variance	Population variance	Cost limit (man-hours)	Estimated optimum sample size ^{2/}
HIGH POPULATIONS							
1 (plots)	9999 ^{3/}	4	--	73.47	61.78	27.0	4.5
2 (subplots)	75	5	0.0667	58.06	-5.68	.7	2.0
3 (trees)	10	5	.5000	637.34	637.34	1.4	10.0
Estimate of mean 35.38				Specified variance of mean 13.03			
MEDIUM POPULATIONS							
1 (plots)	9999	5	--	5.71	-4.50	27.0	1.0
2 (subplots)	75	5	.0667	53.04	29.09	.7	75.0
3 (trees)	10	5	.5000	239.44	239.44	1.4	10.0
Estimate of mean 13.27				Specified variance of mean 1.14			
LOW POPULATIONS							
1 (plots)	9999	7	--	2.13	0.48	27.0	1.2
2 (subplots)	75	5	.0667	8.43	2.41	.7	6.0
3 (trees)	10	5	.5000	60.16	60.16	1.4	10.0
Estimate of mean 8.17				Specified variance of mean 0.69			

^{1/} Precision, 20 percent of mean at $p = 0.05$.

^{2/} Cost is minimized subject to specified variance of the estimated mean.

^{3/} Code for infinite number.

Preliminary stratification of an infested area by an aerial survey should be useful in planning overall allocation of sampling effort. In Oregon and Washington, areas showing different degrees of damage are sketch-mapped according to predetermined standards (Wear and Buckhorn 1955). Although this damage was caused by the previous brood, the differences would furnish broad guidelines for sampling the new generation.

CORRELATION OF SAMPLE ESTIMATES IN SUCCESSIVE PERIODS

Positive relationships between sample estimates at particular life stages are needed to develop a reliable method for predicting damage. Although surveys can utilize a direct relationship between budworm density at a nonfeeding stage and damage, we wanted to include estimates of larvae in buds as part of the predictive process. If it were not possible to predict density of larvae in buds, prediction of damage from an early nonfeeding stage might be suspect. Further, some surveys might be based on sampling larvae in buds if a control decision were not imminent.

The first relationship needed is one between densities of a preceding nonfeeding budworm stage and larvae in opening buds. Stages tested as indicators of larvae in buds were: (1) eggs, (2) hibernating larvae, and (3) pupae of the previous generation. The second and most critical relationship is between density of larvae in buds and damage to current needle growth, to be discussed in the next section.

Egg Stage for Predicting Larvae in Opening Buds

The objective of analysis was to determine whether density of new egg masses or hatched eggs was indicative of density of larvae attacking buds the following spring. The egg stage is a good starting point because hatched egg masses are available for sampling over an extended period.

Methods

Data were obtained from study plots where 10 trees were sampled for egg density and 25 trees for larval density. The sampling unit for egg density was foliage area of a single whole branch removed at midcrown; and for larval density, four 15-inch twigs removed from the lower crown half by use of a pole-pruner. Egg density was expressed as average number of egg masses or hatched eggs per 1,000 square inches of foliage. Larval density was expressed as number of larvae per 100 twigs or per 1,000 buds. Egg mass density at one plot in 1 year was corrected downward because of an unusually high egg sterility. Since normal hatch was 80 to 90 percent, the density at this plot was corrected on the basis of 85-percent hatch.

The degree of association between egg density and larval density was tested by statistical correlation and regression analysis, using a computer program. The basis was 14 plot records when larval density was based on 100 twigs and 12 plot records when it was based on 1,000 buds. At one plot represented by two records, no bud counts were obtained.

Results

Highly significant ($p < 0.01$) linear correlations were obtained between density of larvae per 100 twigs and densities of egg masses and hatched eggs. Correlation coefficients were 0.69 when density of egg masses was the independent variable and 0.71 when density of hatched eggs was the independent variable. There was some statistical evidence in both cases for a curvilinear relationship, but the linear form (figs. 1 and 2) appeared more reasonable. Linear regression equations were as follows:

Larvae per 100 twigs on egg masses per 1,000 square inches of foliage: $\hat{y} = 9.19 + 85.54x$.

Larvae per 100 twigs on hatched eggs per 1,000 square inches of foliage: $\hat{y} = -11.56 + 2.504x$.

Density of egg masses gives similar results to hatched eggs if normal hatching rate (85 percent) and average size of egg mass (42) are considered. However, if oviposition should be abnormal in regard to size of egg mass or percent of eggs hatching, density of hatched eggs should be used for predictions.

A highly significant ($p < 0.01$) linear correlation was obtained between larvae per 1,000 buds and egg mass density ($r = 0.82$). A significant ($p < 0.05$) curvilinear (cubic) correlation was also found, but the linear relationship, $\hat{y} = 25.405 + 25.908x$, appears to be the more reasonable form for prediction. This linear relationship (fig. 3) is better than that found with larvae per 100 twigs (fig. 1). Expressing number of larvae on the basis of buds, rather than 15-inch twigs, results in less variation around regression.

Hibernating Larvae for Predicting Larvae in Buds

In 1950, a study was made to determine relative numbers of hibernating larvae on tree parts of Douglas-fir and the value of samples taken from these tree parts in predicting initial density of spring-feeding larvae. Nondestructive sampling was used so that successive samples could be taken on the same trees. In order to minimize intratree variation, sampling was restricted to the middle third of the crown.

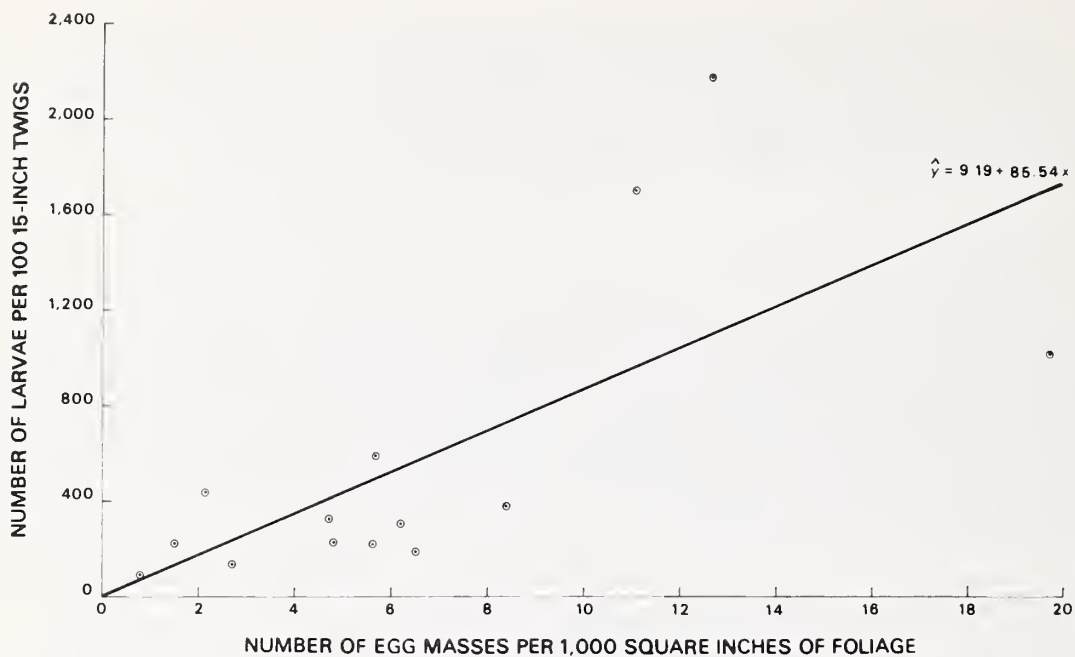


Figure 1.—Linear regression of larval density from twig samples on egg mass density.

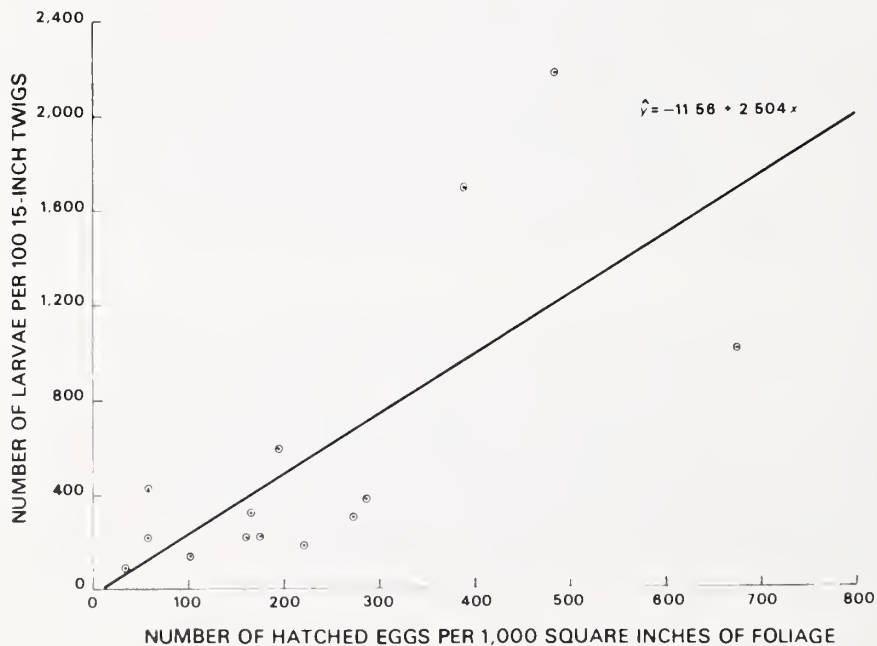


Figure 2.—Linear regression of larval density from twig samples on density of hatched eggs.

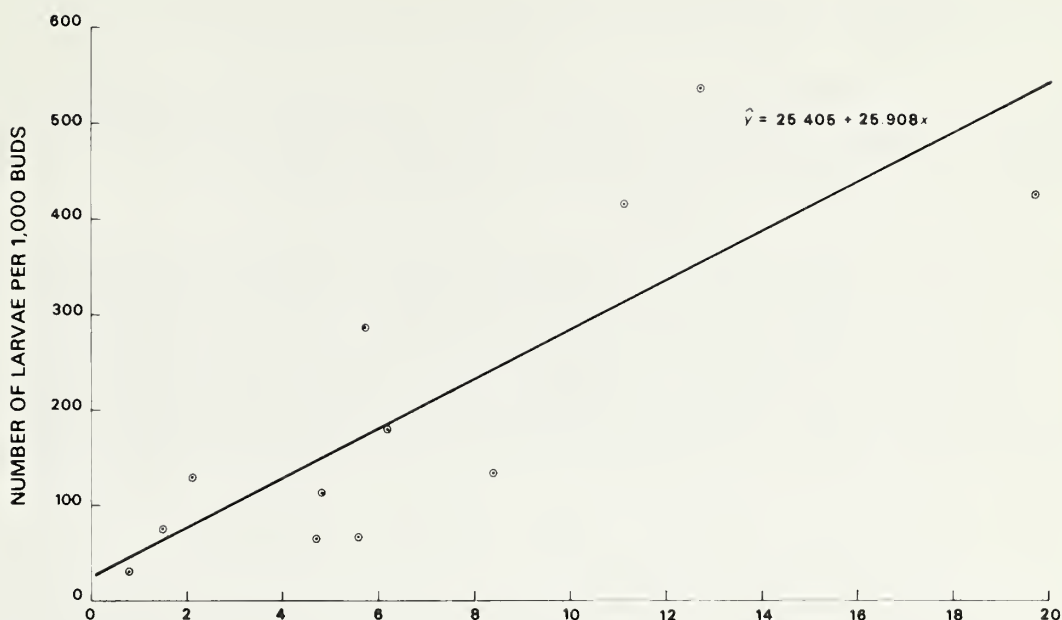


Figure 3.—Linear regression of larval density in buds on egg mass density.

Methods

Samples for hibernating larvae and, later, feeding larvae were taken from the same two midcrown branch whorls, consisting of either six or eight branches on each of 11 codominant trees. Sample trees were scattered at six points over a distance of several miles in the Catherine Creek drainage, near Union, Oregon. The study of distribution on tree parts used only nine of the 11 trees because of a missed observation on two trees.

To obtain estimates of numbers of hibernating larvae, we cut two rectangular bark samples totaling 105 to 226 square inches from opposite sides of the bole at midcrown and removed every second limb in the adjacent two whorls. Sample limbs were divided into bare portions and foliated portions. Bare portions were divided into sections of the main limb and small crooked sections at the base of branchlets. Foliated portions were divided into 15-inch twigs and remaining twigs. Remaining twigs were estimated as one-third of the total foliated length, then discarded. The remaining kinds of material--bole, limbs, branchlet bases, and 15-inch twigs--were boxed separately, and numbers of larvae were recorded as they issued from each box. Total numbers on each

tree part for an average whorl were computed; no conversion was necessary for bare limb portions. Larval densities on bole samples were used to compute numbers of larvae on half the bole area subtending two whorls. Total bole area was computed by averaging the measured circumferences halfway to the whorl above and the whorl below the sampled whorls, and multiplying by the distance between these two points of measurement. Bark area of limbs was determined by similar measurements, for expression of larval density.

We obtained estimates of numbers of feeding larvae, after needle-mining and some bud-mining had occurred, by cutting and examining the remaining branches in the two whorls. Correlations were attempted for (a) number of mined needles and (b) number of feeding larvae with numbers of larvae hibernating on different tree parts. Correlations were also attempted for (a) number of mined needles and (b) number of feeding larvae with hibernating larvae per 100 square inches of bark on boles and limbs. An orthogonal polynomial test was used.

Results

Unfoliated parts of the trees produced the largest numbers of hibernating larvae, as shown by preliminary sampling in 1948.^{4/} Density of lichens on boles and limbs tended to be related directly to numbers of hibernating larvae. On trees having highest populations, limbs produced more larvae than did adjacent bole areas. On trees having relatively low populations, there were usually more larvae on the bole than on the limb sections, and an increased proportion of larvae on foliated parts (table 7). About 79 percent of all larvae at midcrown were found on limb components, which in general agrees with findings by McKnight (1969a) for Douglas-fir in Colorado; he found about twice as many larvae in each crown third on all limb components as on the bole. Densities of hibernating larvae per 100 square inches of bark are shown in table 7.

Limb sections were consistently better than bole samples as indicators of subsequent mined needle or feeding larvae populations. Significant correlations for limb sections--linear, quadratic, or both--were obtained in each case. Correlation coefficients for limb sections were higher than those for bole samples when both showed significant correlations for the same kind of comparison. Estimates of hibernating larvae on bole samples were correlated only with numbers of mined needles and not with numbers of feeding larvae. Correlations for branchlet bases showed the same form and similar coefficients as limb sections. Foliage estimates of hibernating larvae were uncorrelated with mined needles but were correlated with feeding larvae, the latter possibly an artifact due to differential larval survival. Results of correlation analyses are shown in figure 4.

⁴H. L. Haglund. Unpublished report. Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, Portland, Oregon, 1952.

Table 7.--Numbers and density of larvae hibernating on boles and limb sections and numbers subsequently feeding, per average branch whorl at midcrown on Douglas-fir, eastern Oregon, 1950

Tree number	Lichen density	Larvae hibernating on				Whorl total	Density of larvae hibernating on			Index of feeding larvae	
		Boles	Limb sections	Branch bases	Foliage		Tree	Boles	Limb sections	Mined needles	Living larvae
-----Number-----						Per 100 sq. in. of bark			-----Number-----		
1	Heavy	136	322	137	80	675	1	30	69	1,076	780
2	Medium	436	669	386	164	1,655	2	131	99	1,772	940
3	Heavy	330	1,150	466	63	2,009	3	56	86	1,780	360
4 ^{1/}	Light	--	45	27	36	--	4	--	8	608	90
5 ^{1/}	Light	76	7	6	11	100	5	15	1	112	72
6 ^{1/}	Light	--	10	6	17	--	6	--	1	18	70
7	Medium	88	10	4	45	147	7	14	2	128	94
8	Light	57	4	0	24	85	8	19	1	252	85
9	Light	11	28	13	48	100	9	5	6	53	29
10	Light	0	6	1	71	78	10	0	1	64	34
11	(2/)	45	288	97	429	859	11	28	56	1,278	1,228
Total		1,179	2,484	1,110	935	5,708		298	330	7,141	3,782
Percent		20.7	43.5	19.4	16.4	100.0					

^{1/} Not included in total.

^{2/} Lichen density not recorded.

Y	X	Curve form	Boles	Limbs	Branch bases	Foliage
----- Number of -----			Correlation coefficient (R)			
Mined needles	Total hibernating larvae	Linear	0.82	0.90	0.91	NS
		Quadratic	NS	.98	.97	NS
Feeding larvae	Total hibernating larvae	Linear	NS	NS	NS	.85
		Quadratic	NS	.93	.92	NS
Mined needles	Hibernating larvae per 100 square inches	Linear	.80	.97	--	--
		Quadratic	.94	NS	--	--
Feeding larvae	Hibernating larvae per 100 square inches	Linear	NS	.80	--	--
		Quadratic	NS	NS	--	--

Figure 4.--Correlations of estimates of hibernating larvae on different tree parts with estimates of feeding larvae, at midcrown on Douglas-fir trees, eastern Oregon, 1950.

Thus, limb sections appear to be the best sample unit for attempting to predict size of feeding budworm populations. In other regions, bole samples have been tested for predicting defoliation,^{5/} but with unclear results. Regression equations obtained with limb sections are shown below, for possible

⁵R. E. Denton. Two unpublished reports. Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, Coeur d'Alene, Idaho, 1951 and 1953.

application to other stands in the Blue Mountains (\hat{y} = number of feeding larvae, x = number of hibernating larvae).

1. Mined needles on total hibernating larvae (one branch whorl)

Linear $\hat{y} = 257.5 + 1.70 x$

Quadratic $\hat{y} = 114.1 + 4.12 x - 0.0023 x^2$

2. Feeding larvae on total hibernating larvae (one branch whorl)

Quadratic $\hat{y} = 33.3 + 3.60 x - 0.0029 x^2$

3. Mined needles on hibernating larvae per 100 square inches

Linear $\hat{y} = 127.7 + 17.38 x$

4. Feeding larvae on hibernating larvae per 100 square inches

Linear $\hat{y} = 80.1 + 8.79 x$

These results apply specifically to the Blue Mountains and those stands in which trees develop roughened bark on limbs and boles at a relatively early age. Wright et al. (1952) found that, on smooth-limbed trees in the Ochoco Mountains, bole sections yielded significantly more larvae per 100 square inches of bark than did limb sections; Denton (1953) and Terrell (1959) reported the same results for the northern Rocky Mountains. Smooth-bark boles are reported to produce significantly lower densities of hibernating larvae than roughened boles.^{6/} Unfortunately, most studies based on data by control personnel have been preoccupied with tree parts producing the highest number of hibernating larvae, rather than the predictive value of this sampling.

Pupae and Pupal Cases for Predicting Larvae in Buds

To be a good index of numbers of larvae in the buds, numbers of pupae at the time of sampling must be closely related to resulting numbers of female adults that will mate and lay eggs. As with predictions based on the egg stage, consistency in egg production, egg mass size, and percent hatch is required. A more or less consistent survival of small larvae until spring-feeding is also required.

It is not practical to delay sampling of pupae until all moths have emerged, because empty pupal cases gradually break up or fall off the trees. Our timing with 70- to 90-percent emergence appears to be a reasonable compromise involving minimum loss of pupal cases vacated by moths. The apparently sound pupae remaining will include a few containing true pupal parasites, but these represent a very minor error component.

⁶W. E. Cole. Unpublished report. Forest Service, Boise Research Center, Boise, Idaho, 1957.

Methods

Correlations between density of pupae (mostly pupal cases) and density of larvae in opening buds, in the lower crown half, involved two steps. First, correlations were attempted within plots, using numbers per twenty 15-inch twigs recorded at each of the five subplots. Then correlation and regression analyses were attempted for plots, using mean densities of pupae and larvae in buds based on the total 100 twigs. The latter analyses were done by a computer program. The basis was 13 plot records from five standard plots during 1955-58.

Results

Correlations within plots between pupae and larvae in buds were significant in about half the cases. Correlation coefficients were highly significant ($p < 0.01$) in two cases, significant in four cases ($p < 0.05$), and nearly significant in two others. Because each analysis was based on only five comparisons (subplots), further analysis appeared promising.

When plot means were used, highly significant linear and curvilinear correlations were obtained between previous density of pupae and density of larvae in buds ($r = 0.81$, $R = 0.91$). Significant curvilinear effects were quadratic. However, the linear relation (fig. 5), as follows, is surer for prediction.

$$\hat{y} = -62.6 + 5.608x$$

With careful timing and in the absence of unfavorable weather during the moth flight period, density of pupae and pupal cases provides a relatively quick means of predicting density of subsequent larvae in the buds. Use of this method, however, would represent more of a gamble than sampling the egg stage.

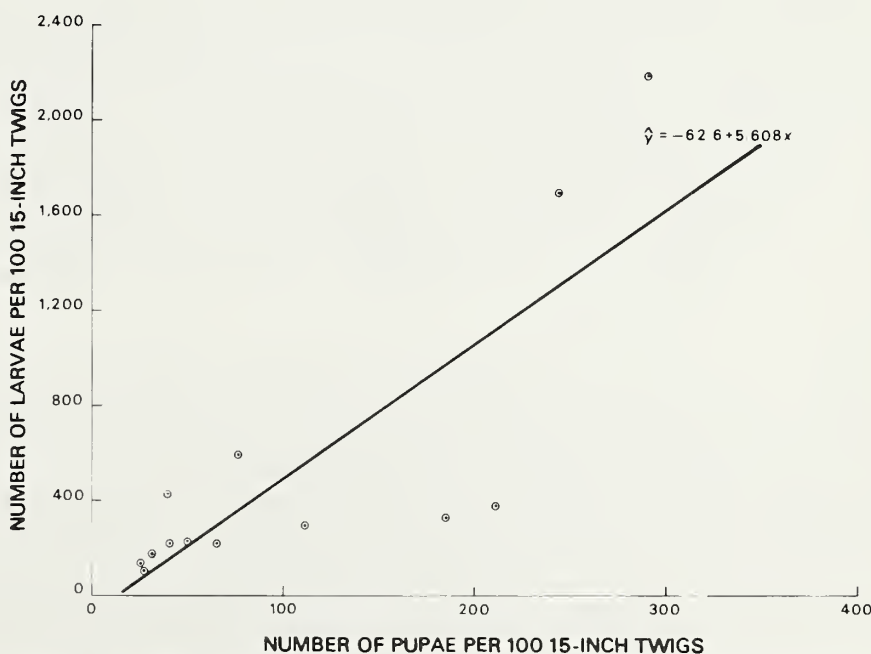


Figure 5.—Linear regression of larval density on previous pupal density.

CORRELATION BETWEEN LARVAL DENSITY AND CURRENT DAMAGE

For a particular tree species in a specific size class, reasonably accurate estimates of density of bud-feeding larvae should provide a good basis for predicting damage that year. Two factors, however, make damage prediction difficult. One is variation in larval survival during the feeding period, among different areas and among years in the same area. The second is quantity and quality of available food, which may vary with time and rate of bud-flushing, normal complement of vegetative buds, size and number of needles per shoot, and incidence of flowering. Obviously, the effects of various factors need to be compensating if a predictive scheme is to be possible.

Damage caused by spruce budworm is usually expressed in terms of percent defoliation of current needle growth. However, as budworm density increases, bud-killing becomes an important symptom of damage. If incidence of bud-killing were a direct reflection of degree of defoliation, usefulness of defoliation for damage predictions would be enhanced. Then, estimates of current defoliation in consecutive years would provide a means of anticipating serious damage (i. e., top-killing) before it occurred and timely control action could be scheduled.

Specific objectives of our studies were to correlate defoliation and larval density on individual trees within plots, in order to determine the relative usefulness of defoliation estimates obtained by fieldglasses and shoot tallies and express a relationship between defoliation and larval density for all plots.

Methods

Fieldglass estimates of current defoliation on individual trees were obtained from five different plots during 1956-59; these constituted eight plot records. Estimates were based on 10, 15, or 25 trees. In obtaining estimates for individual trees, two observers visually divided the tree crown into thirds and made separate estimates for each third. Categories of percent defoliation recognized were as follows: 0-10, 11-25, 26-50, 51-75, 76-90, and 91-100. Midpoints of categories were used in averaging the estimates. A single estimate for the tree was obtained by weighting estimates for crown thirds as follows: lower--5, middle--3, and upper--1. The basis for weighting was developed earlier in this paper.

Shoot tallies were used to estimate defoliation of current growth in three plots where the estimate was expected to exceed 25 percent. Five plot records were obtained, three from one plot during 1955-57, and one each from two plots for a single year. Only the middle crown third was sampled. At the 3-year plot, four 20-inch twigs were removed from each of 15 trees, five at each of three subplots. At the other plots, five to ten 20-inch twigs were taken from each of 10 trees. Shoots were examined in the laboratory. Defoliation on each shoot, estimated to the nearest 10 percent, and number of killed buds were recorded for each tree sample. Numbers of shoots were weighted by their

defoliation category, (0, 10, 20, 30, ... percent) and an average defoliation was determined for the sample. Number of killed buds was used in two ways: 100-percent defoliation, along with shoots completely defoliated, and a separate indicator of damage. Some estimation methods (Terrell 1961, McKnight 1969b) do not take bud-killing into account.

Estimates of defoliation obtained by use of fieldglasses and by shoot tallies were tested for correlation with estimates of larval densities on individual trees within plots. Larval density, the independent variable, was expressed as number of larvae per four 15-inch twigs, although actual samples were two, four, or six twigs per tree. In three cases where total number of buds was counted on sample twigs, larvae per 1,000 buds was also used as an independent variable in correlation analysis.

Mean estimates of defoliation on plots were tested for correlation with mean larval density, and regression equations were obtained using a computer program. The basis was 12 plot records. For low populations, average defoliation was based on fieldglass estimates for individual trees. Also included for analysis were two records in which a general estimate was made for light but generally visible defoliation. For high populations, average defoliation was based on shoot tallies. One record for a medium population was based on fieldglass estimates corrected upward by one defoliation category, based on shoot examinations. Two correlation and regression analyses were run, with different independent variables. In one case, the independent variable was number of larvae per 100 15-inch twigs. In the other, it was number of larvae per 1,000 buds. In most cases, an average number of buds per 15-inch twig for a specific study plot was used to convert from larvae per 100 15-inch twigs to larvae per 1,000 buds. In a few cases, buds had been counted at the time they were examined for larvae, and larval density was based on these counts. Average number of buds or shoots per 100 twigs varied indirectly with stand density, as shown below:

<u>Stand</u>	<u>Plot</u>	<u>Buds per 100 twigs</u>
Closed	Baker	2,100
Closed	Dixie	2,400
Open	Joseph	3,300
Open	Dale	4,070

Results

Reliability of defoliation estimates on individual trees.--Percent defoliation on individual trees as estimated by use of fieldglasses was correlated ($p = 0.05$ or $p = 0.01$) with number of larvae per four 15-inch twigs in half the cases. Correlations were obtained in two or four cases where the larval density was relatively low, and in two of four cases where it was high. Defoliation was usually underestimated in plots having heavy damage; loss of shoots from bud-killing was very difficult to estimate from the ground. In a given year, persistence of dead needles on new shoots made it difficult to estimate either defoliation or shoot reduction.

Use of a different independent variable--number of larvae per 1,000 buds--resulted in a similar incidence of correlations. In one case, comparison with results of analysis using larvae per twig showed a highly significant correlation remained unchanged; in a second case, a nonsignificant correlation became significant; in a third case, a significant correlation became nonsignificant.

Percent defoliation estimated by use of shoot tallies on individual trees was uncorrelated with number of larvae per four twigs in all five cases. Percent defoliation and percent bud-killing on individual trees were also uncorrelated. Nevertheless, the estimates based on shoot tallies undoubtedly gave a more accurate picture of damage at these high-population plots than did fieldglass estimates. Lack of significant correlation probably indicated the need for a larger and better distributed shoot sample.

These findings indicate that properly timed fieldglass estimates will be satisfactory for showing differences in damage between trees, but that shoot tallies are necessary to obtain a realistic picture of damage in plots with high populations. The most efficient method would probably be one which used occasional shoot tallies while fieldglass estimates were being obtained.

*Relationship between larval density and defoliation among plots.--*Correlations between percent defoliation and two expressions of larval density were highly significant. With density expressed as larvae per 100 twigs, the basic regression was linear ($r = 0.88$), but quadratic effects were also highly significant ($R = 0.96$). The curvilinear form,

$$\hat{y} = -9.63 + 0.1108x - 0.0000325x^2$$

is satisfactory for low and medium populations, but it underestimates damage by high populations. A decline in the curve at $y = 85$ reflects the lessened defoliation recorded for 2 years in a plot of open-grown trees; a high complement of shoots reduced the impact of feeding. Nonetheless, the curvilinear relationship appears more realistic than the linear expression,

$$\hat{y} = 12.84 + 0.03814x$$

which appears to compromise the data (fig. 6). The linear form overestimates defoliation for low populations and underestimates it for higher populations.

With density expressed as larvae per 1,000 buds, a high linear correlation ($r = 0.95$) with defoliation was obtained. Curvilinear effects were nonsignificant. The linear form, $\hat{y} = 2.44 + 0.167x$, describes the relationship (fig. 7). Expressing larval density on the basis of a unit number of buds, rather than 15-inch twigs, reduces variation and probably results in better predictions.

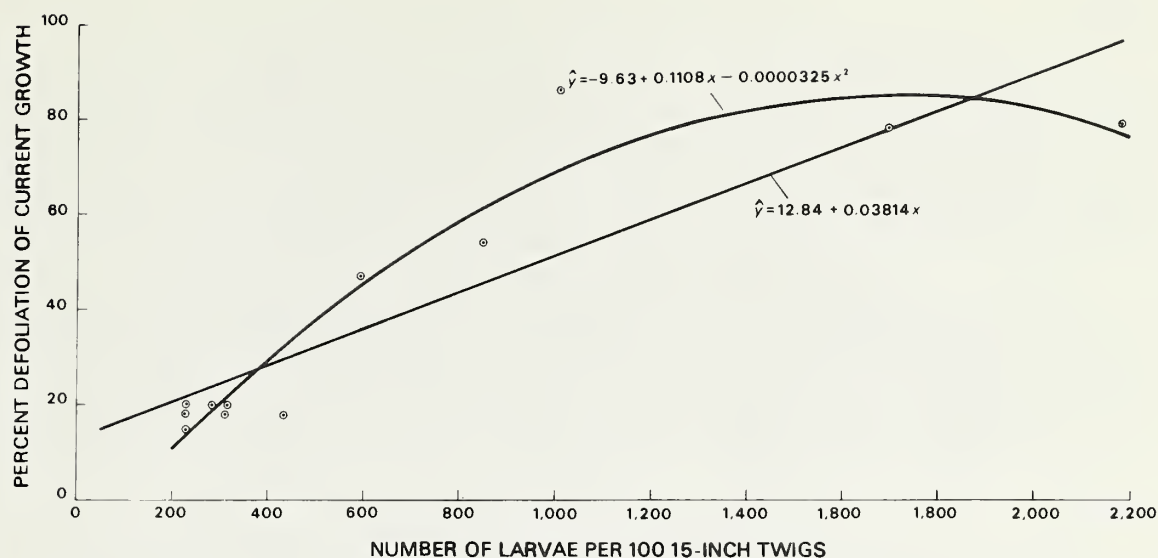


Figure 6.—Linear and curvilinear regressions of defoliation of current growth on larval density from twig samples.

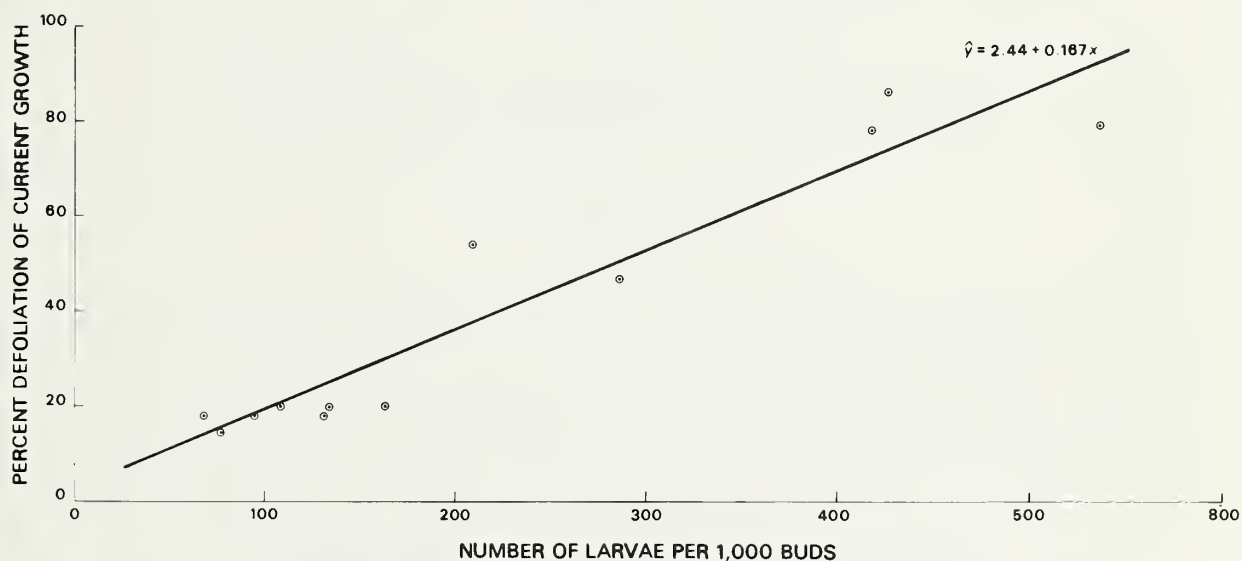


Figure 7.—Linear regression of defoliation of current growth on larval density in buds.

CORRELATION BETWEEN EGG MASS DENSITY AND CURRENT DAMAGE

The relationship between density of egg masses and defoliation of current growth was tested as a final step in developing predictive standards. The estimated defoliation values for given egg mass densities were compared with those resulting from the two-step process, in which egg mass density was correlated with density of larvae in the buds, and the latter correlated with defoliation.

Methods

The degree of association between egg mass density per 1,000 square inches and percent defoliation was tested by statistical correlation and regression analysis. The basis was 10 plot records, which is fewer than used in each step of the two-step process, because most plot sampling was initiated in the spring, at the time larvae were in buds, and because the aerial spraying in 1958 eliminated three major plots where estimates of defoliation were scheduled.

In setting up comparable standards based on the two-step process, regression values of larval density were obtained for specific degrees of defoliation, then egg mass density was calculated for these values of larval density. Two sets of standards were obtained, one expressing larval density on the basis of 100 15-inch twigs and the other on the basis of number of larvae per 1,000 buds.

Results

A highly significant linear correlation ($r = 0.92$) was obtained between density of egg masses per 1,000 square inches and percent defoliation. The relationship is described by the linear form $\hat{y} = 9.02 + 4.588x$ (fig. 8). Curvilinear effects were nonsignificant.

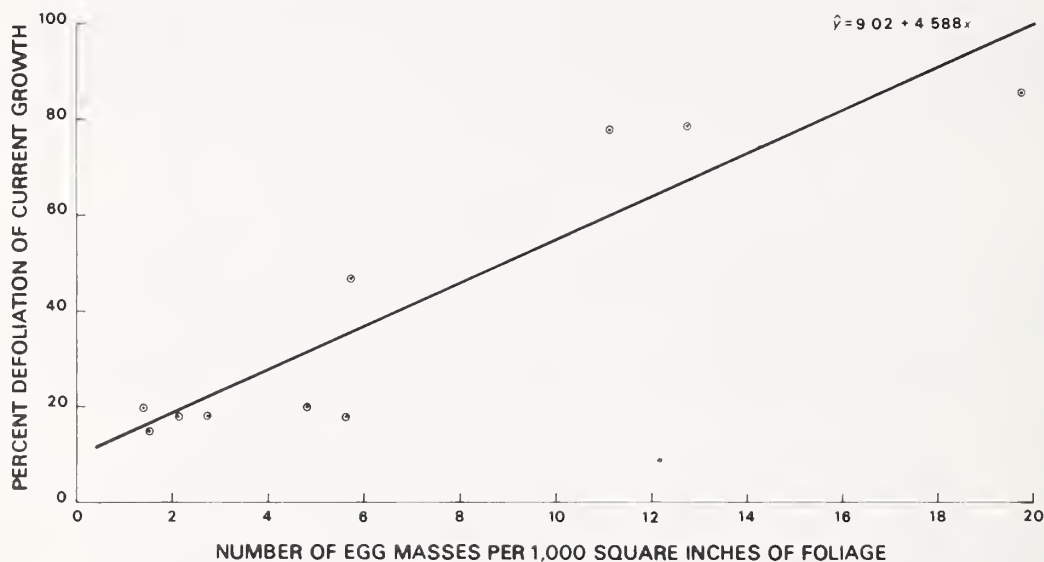


Figure 8.—Linear regression of defoliation of current growth on density of egg masses.

Predictions based on the egg mass density-defoliation relationship according to categories for each variable are shown in figure 9. The two-step method, with larval density based on larvae per 1,000 buds, yields similar predictions. Although the two-step method based on larvae per 100 twigs provides essentially similar predictions for low and medium budworm densities, it is weak on predictions at higher densities (fig. 9).

A. DIRECT PREDICTION OF DEFOLIATION FROM EGG MASS DENSITY

Egg masses <i>Per 1,000 square inches</i>	Defoliation	
	<i>Category</i>	<i>Percent</i>
0 - 1.3	Very light	0- 15
1.4 - 3.5	Light	16- 25
3.6 - 8.9	Moderate	26- 50
9.0-17.7	Heavy	51- 90
17.7 and higher	Very heavy	91-100

B. PREDICTION THROUGH LARVAL DENSITY BASED ON NUMBER OF BUDS

Egg masses <i>Per 1,000 square inches</i>	Larvae <i>Per 1,000 buds</i>	Defoliation	
		<i>Category</i>	<i>Percent</i>
0 - 1.9	0- 75	Very light	0- 15
2.0- 4.2	76- 135	Light	16- 25
4.3-10.0	136- 285	Moderate	26- 50
10.1-19.2	286- 524	Heavy	51- 90
19.3 and higher	525 and higher	Very heavy	91-100

C. PREDICTION THROUGH LARVAL DENSITY BASED ON 15-INCH TWIGS

Egg masses <i>Per 1,000 square inches</i>	Larvae <i>Per 100 twigs</i>	Defoliation	
		<i>Category</i>	<i>Percent</i>
0 - 2.7	0- 239	Very light	0- 15
2.8- 3.9	240- 348	Light	16- 25
4.0- 7.7	349- 669	Moderate	26- 50
7.8 and higher	670 and higher	Heavy	51-100

Figure 9.—Ranges of egg mass density and larval density related to standard categories of defoliation on Douglas-fir, eastern Oregon.

DISCUSSION

These results provide guidance on survey methods to predict western budworm trends and damage over broad areas. The basis is empirical, although life table aspects were necessarily considered in the timing of sampling. Survey efficiency is stressed, but efficiency can no doubt be further increased. One obvious approach is by development of sequential sampling plans (Morris 1954, Waters 1955). Such plans have been developed for egg mass surveys in the central and southern Rocky Mountains by McKnight et al. (1970) and for larval surveys to evaluate control (Cole 1960).

The egg stage and larvae in the buds are particularly suitable for sampling, as others have shown for the eastern budworm. Either stage is useful in biological evaluation if trend information is sought and a control decision not required immediately. A shortcut method of predicting trends by using old egg masses to represent the previous year's new egg masses has been described by Buffam and Carolin (1966); it is not effective in some forest regions.⁷ If a control decision is in the offing, the egg stage is used for predictive purposes as an

⁷Personal communication from W. H. Klein, Forest Entomologist, Intermountain Region, U.S. Forest Service.

index of defoliation; it may also be used to predict density of larvae in buds. Two other life stages--pupae of the previous generation and overwintering larvae of the new generation--offer some promise in predicting density of larvae in buds, and thence defoliation.

Multistage analysis indicates that a cluster design is the best approach in sampling egg and larval populations. This agrees with Morris's (1955) recommendation for sampling the eastern budworm. In 1959, prior to a sophisticated review of our data, we recommended five-tree clusters for egg surveys. Subsequent surveys, mostly in declining infestations, yielded reliable predictions; however, the same amount of effort in rising infestations probably would have been inadequate.

At the stated precision ($SE = 20$ percent at $p = 0.05$), egg-sampling costs are high. At all egg densities, examination of branches is an important cost factor. At low egg densities, sampling is particularly costly because of the number and size of clusters required for the prescribed precision. It is not surprising that Phillips and Proctor (1970), in conducting sampling studies on the oriental fruit moth, decided that sampling eggs was not practicable at low densities. However, in the case of the budworm, a substantial egg-sampling effort would be justified if a sudden rise from a very low population level was indicated by surveys based on beating trees. Some sacrifice in precision could be accepted so long as representation of different stands was maintained. In developing sampling methods for the Douglas-fir tussock moth, Mason (1970) recognized that a relatively low level of precision would have to be accepted in most egg sampling work. Morris (1960) suggests that the advantages of accepting higher sampling errors and expanding the coverage of population densities and environmental conditions have not been fully explored.

Costs of sampling larvae in buds, except for medium populations, are less than costs of egg-sampling. However, surveys for predictive purposes are nullified by the need to complete planning for control before spring. Sampling this stage for trend purposes is limited by the relatively short time available, as compared with egg surveys. However, use has been made for special purposes, such as trends in sprayed areas (Carolin and Coulter 1971) and cooperative ground surveys outside the general zone of infestation. Sampling larvae in buds, with its lower costs, would have particular application in wilderness or other reserved areas. In such areas, control decisions are not at stake, and the usual winter deadline on control planning would not apply. Evaluation of trends would nevertheless be needed, as these could affect subsequent control needs on adjacent lands.

In the previous budworm outbreak in the Pacific Northwest, insurance against unnecessary spraying was sought by qualitative assessment of limb and bole samples for hibernating larvae. These efforts required considerable labor, and results were often questionable. Aside from the problems in sampling

design, handling and methods of rearing the samples were recognized as a possible source of error (Wright et al. 1952). Now, new techniques for sampling overwintering larvae of the eastern budworm, based on washing larvae from their hibernacula, have been described by Miller and McDougall (1968) and Miller et al. (1971). These should be tested on the western budworm.

The predictive relationships based on an initial estimate of egg mass density are specifically for Douglas-fir in the Blue Mountains. Relationships between density of egg masses and density of larvae in the buds imply a particular average number of eggs per mass, percent of eggs hatching, and survival from eclosion until attack of buds the following spring. Differences among western regions should result in different predictive relationships, and egg mass size is known to vary among western regions (Carolin and Honing 1972). Eastern Oregon egg masses are large, averaging 40-42 eggs for all infestations, and Silver (1960) recorded an average of 44.8 for an infestation in southern British Columbia. McKnight et al. (1970) developed predictive standards for central and southern Rocky Mountains, utilizing sequential sampling to define four infestation classes. Average egg mass size was not defined but presumably was consistent among areas.

Percent defoliation of current growth is a convenient means of expressing annual damage, but incidence of bud-killing is undoubtedly a more important damage factor. Top-killing and branch-killing, used as indicators of cumulative damage by Williams (1967), are believed to be a direct result of continued bud-killing. The sequence of damage effects leading to serious growth impact of host trees needs to be documented as a basis for survey evaluations.

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Keywords: Western spruce budworm, *Choristoneura occidentalis*, Douglas-fir, defoliation.

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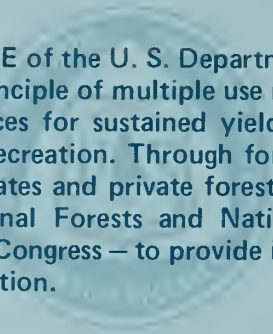
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